Isolation and Identification of Fungal Species Associated With the Spoilage of Some Selected Edible Fruits in Iree Town Of Boripe Local Government, Osun State, Nigeria

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ABSTRACT:- Four Spoilage fruits were investigated in this study. They were pawpaw (Carica papaya), Pineapple (Ananas comosus), Orange (Citrus sinensis) and Tomato (Lycopersicon esculentum), which were collected from a market at Iree town. All the fruits collected showed signs of spoilage by fungal species. The fungal species isolated were Aspergillus flavus, Rhizopus stolonifer, Aspergillus niger, Candida tropicalis, Phytophthora sp., Fusarium oxysporum and Mucor sp. Among all, Aspergillus species had the highest rate of occurrence followed by Rhizopus and Candida species while Phytophthora sp., Mucor sp. and Fusarium sp. were the least. Finally, the pathogenicity tests showed that all the fungi isolated were pathogenic to all the fruits.

Keywords:- Spoilage, fruits, fungal species, pathogenicity.

I. INTRODUCTION

Fruits and vegetables generally give the body every necessary vitamins, fats, minerals and oil in the right proportion to maintain growth and development on humans, (1).

Fruits and vegetable however, have serious challenges to their existence and these include changes in climatic condition, pests, inadequate rainfall and fungal attack (1). Over the years, there has been an increase in the need to identify and isolate the fungal associated with their spoilage.

Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic, these changes may be accompanied by alteration in taste, smell, appearance or texture (7). Numerous microbial defects of agricultural crops are characterized by the types of microorganism responsible for the deterioration, the process of infection in the case at fungal invasion follows the development of fungal penetrating structure (appresorium) (7). Colonization of fungi is a critical phase in the microbial spoilage of post harvested fruits. The colonization process involves the ability of the microorganism (fungi) to establish itself without the produce (host). This is initiated when fungi depolymerises certain specific cell wall polymers (such as protopctin, the cementing substance) of the produce (10).

The contamination of fruit and vegetables by fungi could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (4).

Fungi affecting Tomatoes (Lycopersicum esculentum) includes Fusarium oxysporium, Fusarium stolonifer, Aspergillus niger and Rhizopus stolonifer. They are responsible for tomato soft rot, as was isolated by (6). Result on the percentage incidence and rot shows that Rhizopus stolonifer caused the greatest rot on tomato fruit. A lot of breeding works have been carried out on tomato up to the point where we now have tomato hybrids that could withstands adverse environmental condition and resistant to diseases and pests (3).

The preponderance of the isolated moulds from orange (Citrus sinensis) belongs to Aspergillus sp., and this confirms their prevalence in foods and fruits exposed to tropical humid climate thus constituting potential health risks to consumers of this fruit and its by-products (5). The aim of this study is to isolate
and identify those fungi associated with source fruits which eventually caused their spoilage and result to economy loss.

II. MATERIALS AND METHOD

Pawpaw, pineapple, orange and tomato fruits were purchased from a market from Iree town, in Boripe Local Government of Osun State. A total of twenty four fruits comprising of six fruits from each fruit were purchased. Among each of the six fruits, there were three affected, and three healthy looking fruits. Thus, a total of twelve rotten fruits are involved. The fruits were put in 90% ethanol sterile polythene bags and transported to the laboratory for analysis.

Isolation of fungi from each of the blemished fruits was carried out using the method of (1);(2). 3-5cm of the tissues from the margins of the rotten fruits were cut with a sterile; scalped and placed on the prepared Sabouraud Dextrose Agar in petridishes and incubated at 28±1°C for 5 days. The detected fungi were carefully subcultured on Sabouraud Dextrose Agar in plates and slants. The plates and slants were grown for seven days in an incubator at 28°C.

Fungal isolates from plates were prepared into mounts on microscopic slides and examined under the microscope for comparison of fungal morphology with description given by Samson and Reenen-Hoekstra (9).

III. PATHOGENICITY OF ISOLATED FUNGI

Three healthy fruits from each sample were surface sterilized with 90% ethanol, and incisions were made on them using a sterile 4mm cork borer, similar sterile cork borer was used to cut pellets of Agar containing the cultures of fungal mycelia of the isolates. The fungi were inoculated onto the hole created on the healthy fruits in a laminar flour chamber. The inoculated wound was sealed with petroleum jelly. Two controls with incision but not inoculated were established, were placed in a clean polythene bag (one fruit per bag) each moistened with-wet balls of absorbent cotton wool to create a humid environment and incubated at 28°C for 5 days. After 72 hours, the incubated fruits were observed for symptom development. The causal agents were pre-isolated from the infected fruits and compared with the original isolates.

IV. RESULTS

Seven fungi species, Aspergillus sp., Rhizopus sp., Candida sp., Fusarium sp., Phytophthora sp. and Mucor sp. were isolated from a total of twelve fruits. Aspergillus flavus was isolated from pawpaw (Carica papaya) and pineapple (Ananas comosus), while Aspergillus niger was isolated from orange (Citrus sinensis), Rhizopus sp. was obtained from pawpaw (Carica papaya) and tomato (Lycopersicon esculentum) Candida sp was isolated from orange (Citrus sinensis) and tomato (Lycopersicon esculentum) Fusarium sp. and Mucor sp. were isolated from tomato (Lycopersicon esculentum) and Phytophthora sp. from pineapple (Ananas comosus) (table 1).

The frequency of occurrence shows that Aspergillus sp. had the highest frequency in which it occurred in five out of the twelve fruits tested (Table 2). Pathogenicity tests showed that the isolated organisms were pathogenic, because they were able to produce the same spoilage signs in the healthy fruits into which they were re-inoculated (Table 3).

<table>
<thead>
<tr>
<th>Fruit Sample</th>
<th>Spoilage</th>
<th>Macroscopic Examination</th>
<th>Microscopic Examination</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pawpaw (Carica papaya)</td>
<td>Sunken large depression</td>
<td>White colony with light yellow green, become dark yellow-green</td>
<td>Radiate conidial head, coarsely roughened vesicle conidia and flask shaped pholides chains of rough</td>
<td>Aspergillus flavus</td>
</tr>
<tr>
<td>Pawpaw (Carica papaya)</td>
<td>Wrinkled appearance with depression soaked with water</td>
<td>Whitish colony later becoming brown-black</td>
<td>Non-septate mycelia, with sporangiousphore, ovoid in shape with sub-globe columella</td>
<td>Rhizopus stolonifer</td>
</tr>
<tr>
<td>Pineapple (Ananas comosus)</td>
<td>Sunken large depression</td>
<td>White colony with light yellow-green later becoming dark-yellow-green</td>
<td>Radiate conidial later split to loose columns. Rough conidia bone on vesicle</td>
<td>Aspergillus flavus</td>
</tr>
</tbody>
</table>

TABLE 1: Identification of fungal isolates from fruit samples with different spoilage patterns.

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**TABLE 2: Frequency of occurrence of each isolate in fruit samples**

<table>
<thead>
<tr>
<th>Fruit Sample</th>
<th>Total Number of Samples</th>
<th>Fungal Species</th>
<th>Percentage Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pawpaw (Carica papaya)</td>
<td>2</td>
<td>A. flavus, A. stolonifer</td>
<td>66.7%, 33.3%</td>
</tr>
<tr>
<td>Pineapple (Ananas comosus)</td>
<td>1</td>
<td>A. flavus, Candida tropicalis, Phytophthora sp.</td>
<td>33.3%, 33.3%, 33.3%</td>
</tr>
<tr>
<td>Orange (Citrus sinensis)</td>
<td>1</td>
<td>C. tropicalis, A. niger</td>
<td>33.3%, 66.7%</td>
</tr>
<tr>
<td>Tomato (Lycopersicon esculentum)</td>
<td>1</td>
<td>Rhizopus stolonifer, Mucor sp., Fusarium oxysporium</td>
<td>33.3%, 33.3%, 33.3%</td>
</tr>
</tbody>
</table>

**TABLE 3: Pathogenicity Tests**

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Fruit Inoculums</th>
<th>Spoilage Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus</td>
<td>Pawpaw, Pineapple</td>
<td>Sunken spots, Sunken depression</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Orange</td>
<td>Dark brown discoloration with sunken spots</td>
</tr>
<tr>
<td>Rhizopus stolonifer</td>
<td>Tomato, Pawpaw</td>
<td>Water soaked wrinkled appearance</td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>Tomato</td>
<td>Water soaked, Appearance with whitish depression</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>Orange, Pineapple</td>
<td>Fruit become spongy with prominent of gas and white deposits appearance</td>
</tr>
<tr>
<td>Fusarium oxysporium</td>
<td>Tomato, Pineapple</td>
<td>Whitish-pink mycelia growth, Whitish foamy-like growth with sunken depression</td>
</tr>
</tbody>
</table>

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V. DISCUSSION

Aspergillus sp. and Rhizopus sp. isolated from pawpaw in the study responsible for the soft rots of pawpaw. This is also reported by (2). Aspergillus niger and Candida tropicalis were found responsible for the deterioration of orange, this is in correlation with the work of (5) who reported that Aspergillus sp. is the predominant organism associated with the spoilage of orange.

The isolation of Phytophthora sp., Aspergillus flavus and Candida tropicalis from pineapple is in correlation with the work of (4) who reported that Aspergillus sp. and Candida sp. are responsible for the rotting of pineapple.

The isolation of Fusarium organism, Rhizopus stolonifer and Mucor sp. from tomato revealed and supported the work of (4);(3);(8), who reported that Fusarium sp. and Rhizopus stolonifer are responsible for the soft rot of tomato.

Colonization of fruits and vegetable by the invading microorganism is a critical phase in the microbial spoilage of produce.

Also, the prevalence of fungi as the spoilage organisms of fruits and vegetables is due to a wide range of factors which are encountered at each stage of handling from pre-harvest to consumption and is related to the physiological and physical condition of the produce as well as the extrinsic parameters to which they are exposed (4).

Efiuvwevwere,(4) also reported that high moisture and relative humidity led to greater fungal growth in farm produce which tends to lower the storability of fruits and vegetables.

VI. CONCLUSION

Since fruits and vegetables have been very important in human nutritional qualities, this then calls for proper handling of the produce from the pre-harvest to consumption.

All the fruits are advised to be eaten fresh or cooked either to avoid long term exposure to spoilage. Also, the high moisture content of fruits and vegetables will be a serious limiting factor in their preservation. Finally, the farmers who harvest the fruits into bags for transportation, the marketers and consumers should take necessary precautions in preventing contamination and also try to create an environment that would discourage the growth or multiplication of microorganisms.

All the above will help to reduce the risk to poisoning by aflatoxin and other mycotoxins which are normally produced by those fungi isolated in this study. Hence, this is a great concern to all.

REFERENCES